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(54) **Monoclonal antibodies to Fc receptors for immunoglobulin G on human mononuclear phagocytes; bifunctional antibodies; target specific effector cells; targeted macrophages; and immunoassays.**

(57) A human Fc receptor-specific monoclonal antibody is disclosed together with its mode of preparation. Binding of the antibody to Fc receptor is not blocked by human immunoglobulin G. The antibody binds to the high affinity Fc receptor for IgG on human monocytes at a receptor binding site distinct from the ligand binding site for Fc.

A bifunctional antibody or a heteroantibody has an antigen binding region derived from an anti-Fc receptor antibody and an antigen binding region specific for a target epitope or cell; such antibody may target a macrophage when it is bound to surface Fc receptors of the macrophage.

A target-specific effector cell expresses receptor for the Fg portion of IgG, has one antigen binding region derived from an anti-Fc receptor antibody and another specific for a target cell, and the aforesaid bifunctional or hetero-antibody is bound to the Fc receptor of the effector cell; such effector cell can be used in the therapy of cancers, allergies, infectious and autoimmune diseases, and in immunoassays.

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animal is immunized with the purified receptor protein and antibody-producing cells are harvested from the animal and fused with a myeloma cell or other immortalizing cell to produce hybridomas. The hybridomas are cloned and clones are selected for production of antibody to Fc receptor which is not blocked by human IgG.

The selection of antibody which binds to the Fc receptor through its antigen binding region (distinct from the Fc portion of the antibody) is complicated by the fact that the Fc portion of IgG of the animal species may bind human Fc receptor. For example, two of the four murine IgG subclasses -IgG2a and IgG3 - bind to the high affinity human Fc receptor via their Fc portion. In such instances selection can be facilitated as follows: After initial screening of hybridomas for production of Ig which binds the receptor, hybridomas which produce antibody of the subclass which is bound via its Fc region by the human Fc receptor, are eliminated from consideration. The remaining hybridomas are evaluated for production of antibody which binds Fc receptor independently of their Fc portion.

The anti-Fc receptor antibody of this invention can be used to produce target-specific effector cells for treatment of cancer, allergies, and infectious and autoimmune diseases. Antibody specific for a target cell (targeting antibody) can be linked to the Fc receptor of effector cell through the Fc-specific antibody of this invention. The linkage mediated by this anti-Fc receptor antibody is not disruptable by IgG because binding the receptor does not involve the Fc portion of the antibody.

For the purpose of targeting effector cells, a bifunctional antibody (used herein to mean a single antibody or antibody fragment with a dual binding specificity) or a heteroantibody (used herein to mean an aggregate of two or more antibodies (or antibody fragments) each antibody having a different specificity) can be produced. In general, the bifunctional antibody or heterantibody comprises:

a. at least one antigen binding region derived from an anti-Fc receptor antibody whose binding to human Fc receptor is not blocked by human immunoglobulin G; and

b. at least one antigen binding region specific for a target cell.

The binding of bifunctional or heteroantibody to the effector cell results in a targeted effector cell i.e., an effector cell with attached bifunctional or heteroantibody containing antigen binding regions which are specific for a desired target cell. The targeted effector cells can be used to bring about antibody dependent cell mediated cytolysis (ADCC) of the target cells in vivo.

The target cell can be a cancer cell or other cell whose elimination would be beneficial to the host, for example, an auto-antibody producing cell found in autoimmune diseases, or an IgE producing cell found in allergies. The target cell specificity of the bifunctional antibody or the hetero-antibody is derived from a targeting antibody i.e., an antibody specific for a target cell-associated or target cell-specific antigen. The use of the Fc specific antibody of this invention provides for attachment of the targeting antibody to monocyte effector cells by a linkage which is not disrupted by phsiological levels of immunoglobulin G encountered in vivo. Thus, the targeted effector cells can be given in vivo without loss of effector cell specificity due to IgG competion for Fc receptor sites.

The anti-FcRI antibody of this invention has other therapuetic applications as well as several diagnostic applications. The antibody can be used as a targeting antibody to target FcRI-bearing cells. The antibody can also be used to induce capping and removal of Fc receptors on monocyte or other cells. Diagnostic applications of the antibodies include their use in assays for FcRI receptor levels and assays for substances that influence FcRI receptor levels.

The invention will now be described in more detail in the following description, which is given by way of example only, and which is to be read in conjunction with the accompanying drawings, in which:

Figure 1 shows SDS-PAGE of affinity adsorbed lysates of surface radioiodinated U937 cells.

Figure 2 shows SDS-PAGE analysis of affinity adsorption with ligand or with mab 32 after pre-clearing U937 lysates with ligand or with mab 32.

Figure 3 shows the results of isoelectric focussing of p72 purified either with ligand or with mab 32.

Figure 4 shows that human IgG does not interfere with the binding of Mab 32 to U937 cells, but blocks, almost completely, the binding of the mouse IgG2a myeloma UPC-10.

Figure 4b shows that human IgG does not interfer with the binding of Mab 32, 22, 44, 62 and 197 to U937 cells, but blocks almost completely the binding of mouse IgG2a UPC-10; and the increased binding of Mab 32, 22, 44, 62 and 197 to IFN-gamma treated U937 cells.

Figure 5 shows the fluorescence intensity of cells stained with mab 32.

Figure 6 shows the cytotoxicity of chicken red blood cells (cRBC) by IFN-gamma treated U937 cells mediated by the heteroantibody Mab32 x Fab anti-cRBC.

Figure 7 shows cytotoxicity of cRBC by interferon-gamma treated and untreated U937 cells.

Figure 8 shows cytotoxicity of chicken cRBC by interferon-gamma treated and untreated human peripheral blood monocytes.

The anti-Fc receptor antibody of this invention can be used to produce target-specific effector cells i.e. effector cells which are capable of recognizing and binding to a target cell and exerting their effector function. It provides a means for attaching to an effector cell an antibody or antibody-binding fragment directed against a target cell. The attachment is not disruptable by physiological concentrations of IgG because the anti-Fc antibody which mediates the attachment binds the receptor through its antigen-binding region. Effector cells, such as macrophages, targeted in this way can be employed to bring about antibody-dependent cell-mediated killing of target cells.

To target effector cells, bifuctional antibodies or heteroantibodies are employed. These antibodies have dual antigen binding specificity -one specificity for the Fc receptor (preferably the high affinity Fc receptor) and one specificity for an epitope of the target cell. The Fc receptor specificity mediates linkage to the effector cell through a known cytotoxic trigger molecule. The target cell specificity provides for recognition and binding to the target cell.

Bifunctional antibodies are single, divalent antibodies which have two different antigen binding sites. Bifunctional antibodies for targeting have one binding site for Fc receptor and one binding site for a target cell epitope.

Hetereoantibodies are two or more antibodies or antibody binding fragments (Fab) linked together, each antibody or fragment having a different specificity. Heteroantibodies for targeting comprise an antibody (or antigen binding fragment specific for Fc receptor, coupled to an antibody (or antigen binding fragment thereof) specific for a target cell epitope.

Bifunctional antibodies can be produced by chemical techniques (see e.g., D. M. Kranz et al., Proc. Natl . Acad. Sci. USA 78,5807 (1981)) by "polydoma" techniques (See U.S. Patent 4,474,893, to Reading) or by recombinant DNA techniques. heteroantibodies can be prepared by conjugating Fc receptor antibody with antibody specific for an epitope of a target cell. A variety of coupling or cross-linking agents can be used to conjugate the antibodies. Examples are protein A, carboiimide, and N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP). SPDP is the preferred agent; procedures for crosslinking antibodies with this agent are known in the art. See e.g., Karpovsky et al., (1984) J . Exp. Med. 160:1686; Liu, M.A. et al., (1985) Proc. Natl. Acad. Sci USA 82:8648.

Target cells are cells whose elimination would be beneficial to the host. One important type of cell is a tumor cell. Effector cells can be targeted with bifunctional or heteroantibody having specificity for FcRI and specificity for a tumor associated or tumor specific antigen.

Antibodies with a desired tumor specificity for production of bifunctional antibody or hetero-antibody can be produced or can be selected from available sources. Monoclonal antibodies against tumor-associated antigens can be made by the methods of Koprowski et al., U.S. Patent 4,172,124. Many suitable anti-cancer antibodies are presently available.

Specific anti-tumor antibodies would include, but not be limited to:

Antibody	Specificity
AML-2-23, PM-81, PMN-6, PMN-19	Myeloid Leukemia
SCCL-1, SCCL-175	Small Cell Carcinoma of the Lung
OC1-25, OVCT-3	Ovarian Carcinoma
COL-1, COL-2, COL-3, ... COL-13	Colon Carcinoma

In addition to tumor cells, the effector cell can be targeted against auto-antibody producing lymphocyte for treatment of autoimmune disease or an IgE-producing lymphocyte for treatment of allergy. The target can also be microorganism (bacterium or virus) or a soluble antigen (such as rheumatoid factor or other auto-antibodies).

Effector cells for targeting are human leukocytes, preferably macrophages. Other cells would include monocytes, IFN-gamma activated neutrophils, and possibly IFN-gamma activated natural killer (NK) cells and eosinophils. Macrophages can be treated with IFN-gamma before targeting, to increase the number of Fc receptors for attachment of the targeting antibody or heteroantibody. The effector cells may also be activated before targeting by other cytokines such as tumor necrosis factor, lymphotoxin, colony stimulating factor, and interleukin-2. If desired, effector cells for targeting can be obtained from the host to be treated.

from Cappel, West Chester, PA, unless indicated otherwise; RPMI 1640 from Gibco, Grand Island, NY, and from K C Biologicals, Lenexa, KS; Fetal bovine serum (FBS) from Sterile Systems, Logan UT; and a mixture of low molecular weight markers from Biorad, Richmond, CA. Recombinant gamma interferon was kindly donated by Genentech, South San Francisco, CA. 1,25-dihydroxycholecalciferol ($1,25(OH)_2D_3$) was a gift from Hoffman LaRoche, Nutley, NJ. Other chemicals were of analytical grade and were obtained commerically.

NP40 lysis buffer contained 1% NP40, 110mM NaCl, 10mM EDTA, 2mM PMSF, 10ug/ml pepstatin, 10ug/ml chymostatin, 10ug/ml leupeptin and 10ug/ml antipain in 20 mM Tris buffer, pH 7.1. Krebs Ringer phosphate buffer with glucose (KRPglu) consisted of 135mM NaCl, 5 mM KCl, 1.2mM MgSO4, 1mM $CaCl_2$, 4.3mM glucose in 10mM sodium phosphate buffer, pH 7.4. Phosphate buffered saline 9PBS) was 145mM NaCl in 20mM phosphate buffer, pH 7.0. PBS-K contained 130mM NaCl and 5mM KCl in 10mM phosphate buffer, pH 7.4.

Antibodies

The monoclonal antibody against the high affinity FcR (herein designated mab 32 and when subcloned, 32.2), was prepared as follows: A partially purified detergent lysate of the high affinity FcR from U937 cells was obtained in a manner similar to a published method (See Anderson, C.K., et al., (1984) J. Immunol. 134:465-470). U937 cells were lysed in 1% NP40 and the lysate was allowed to incubate with Sepharose hIgG for 8 hours. The adsorbent was washed thoroughly and was eluted with 0.5M acetic acid in 30mM octylglucoside. The eluate was promptly neutralized with 2M Tris and the amount of protein eluted was determined by a Folin assay (Peterson, G.L. (1977) Anal. Biochem. 85: 346-356). The tubes containing the bulk of the protein were pooled, concentrated by vacuum dialysis using an Amicon YM-10 filter and a Minicon apparatus to 0.5ml and emulsified with an equal volume of Freund's adjuvant, either complete for the first injection or incomplete for subsequent ones. A mouse was immunized intraperitoneally 4 times at roughly 4 week intervals, the last 2 immunizations using antigen derived from U937 cells cultured 72 hours in IFN-gamma, 100 IRU/ml, to increase the yield of FcR (Guyre, P.M., et al. (1983) J. Clin. Invest. 72:393-397). Five days following the last immunization, the splenocytes were fused with cells of the NSI myeloma line by standard techniques (Kohler, G. and Milstein, C. (1975) Nature, 256:495; Ball, E.D., et al., (1982) PNAS 79:5374-5378). Supernatants of the hybrids were screened for their ability to bind to U937 cells by an indirect immuno-fluorescence assay using a flow cytometer. Chosen hybrids were cloned by limiting dilution, rescreened and expanded either in culture or in ascites fluid. The protein from clone mab 32 was found to be an IgG1 antibody by an immunoblot assay using isotype-specific antisera. IgG of this clone was precipitated from ascites by making the solution 40% in ammonium sulfate. The precipitate was redissolved and dialyzed against 20mM Tris buffer, pH 8.6. High performance on exchange chromatography (HPLC) was carried out on a semi-preparative PROTEIN-PAK-5PW (Waters, Milford, MA) column. The initial eluting buffer was 20mM Tris, pH 8.6 delivered by pump A. 20mM Tris, 0.3M NaCl, pH 8.6 was delivered by pump B. A sixty minute linear gradient, 0-100% B, at a flow rate of 8 ml/min was used for elution. The main peak corresponding to IgG was pooled. For some experiments the purified IgG was passed over a SepharoseProtein A column to remove traces of IgG2a to less than 0.005%. Pepsin digestion of whole antibody was performed essentially as described by Parham (See Parham, P. (1983) J. Immunol. 131: 2895-2902) except that the digestion time was 3 hr and the pH 3.6. F(ab')2 was purified by high performance gel filtration chromatography using a TSK 250 column (Biorad). Fab' was made from F(ab')2 by reducing with 1mM dithiothreitol for 1 hr at room temperature and alkylating with an excess of iodoacetamide. The Fab' was purified by HPLC using the TSK 250 column.

The preparation of mab 22, mab 44, mab 62 and mab 197 were as above, except that for mabs 22, 44 and 197 the immunogen was IFN-gamma-and dexamethasone activated U937 cells. All procedures and preparations were the same as for mab 32.

The preparation and properties of monoclonal IV3 have been described. See e.g. Looney, R.J. et al. (1986) J. Immunol. 136 :1641-1647. IV3 was used as supernatant fluid from the culture of cloned cells. Fab fragments of IV3 were prepared as described Looney, R. J. et al. IgG or IgM fractions of murine monoclonal antibodies or myeloma proteins MOPC 141 (IgG2b), anti-Vk3b (IgG2b), P3 (IgGl), AML-2-23 (IgG2b), MY23 (IgG1), RPC5 (IgG2a) and MMA (IgM) were purified from ascites fluids by on exchange chromatography unless otherwise indicated. In some cases, the supernatant fluid of cloned hybridoma cells was used. Gap8.3 ascites fluid was donated by Dr. Christopher Francz, Department of Pediatrics, University of Rochester. MY7 was purchased from Coulter, Hialeah, FL. Leu-M3, an anti-monocyte monoclonal antibody, was obtained from Becton-Dickinson, Mountain View, CA.

Binding and Inhibition Experiments

A human IgG1 myeloma protein (Arr) and the IgG fraction of mab 32 were radioiodinated by the chloroglycouril method to a specific activity of 1-5uCi/ug. Preliminary experiments established that equilibrium and saturation were achieved with 4 x 10^7 U937 cells/ml after 2hr at 0°C at 0.3 ug/ml for mab 32 and 1.5ug/ml for hIgG1. Inhibition of the binding of both ^{125}I-hIgG and ^{125}I-mab 32 to U937 cells by unlabeled preparations of both hIgG1 and mab 32 was evaluated by incubating the radioligand or radioantibody with cells in the presence of titered amounts of unlabeled antibody or ligand under the above conditions. Cell-bound radioactivity was separated from free by centrifuging triplicate 50ul portions of the cells suspension through an oil mixture as described. Anderson, C. L. and Abraham, G. N. (1980) J. Immunol. 125:2735-2741. Nonspecific binding was measured in replicate samples containing a great excess of ligand (3mg/ml) or antibody (333ug/ml). Percent inhibition was calculated as described. Anderson, C. L. and Spiegelberg, H. L. (1981) J. Immunol. 126 :2470-2473/

Fluorescence and Flow Cytometry

One million cells were incubated for 2 hr at 4°C in 50ul of antibody (either mab 32 or IV3 supernatant or 10ug/ml solutions of purified IgG fractions of ascites fluid) diluted in RPMI 1640 growth medium containing 4mg/ml hIgG to block nonspecific binding. The cells were washed x3 in PBS containing 0.1% NaN_3, were resuspended and incubated for 2 hr at 4°C in 50ul FITC anti-mIgG (TAGO, Burlingame, CA, or Boehringer-Mannheim, Indianapolis, IN), and were washed a final 3 times. Stained cells were analyzed on an Ortho 50H Cytofluorograf flow cytometer using argon lasers at 300mW or 500mW power. Green fluorescence was collected through a 525 nm bandpass filter on 10,000-50,000 cells gated for low angle light scatter (to exclude erythrocytes, platelets, dead cells and debris) and 90°C light scatter (to distinguish monocytes or neutrophils from lymphocytes). See Salzman, G. C. et al. (1975) Acta Cytol . 19:374. The 90° light scatter signal characteristic of monocytes was determined by separately staining an aliquot with anti-monocyte antibody Leu-M3. Based on these data, gates for 90° light scatter were adjusted so that the green fluorescence signal of monocytes and lymphocytes in mononuclear cell suspensions could be separately collected. Green fluorescence was collected as a linear signal.

Results

The strategy for the development of monoclonal antibodies against the high affinity 72kDa FcR had to deal with the obsevation that two of the four murine IgG subclasses, IgG2a and IgG3, bound with high affinity to this FcR. Thus, any assay for FcR binding would register all antibodies of these two subclasses. Our protocol, therefore, called for immunizing a mouse with partially purified FcR from U937 cells, screening the hybrid supernatants for an Ig capable of binding U937 cells, eliminating from further consideration IgG2a and IgG3 antibodies, and evaluating the remaining antibodies for their capacity to immunoprecipitate 72kDa surface molecules. (Additional monoclonal antibodies of this specificity (designated mab 22, mab 44 and mab 197) were prepared using whole U937 cells.)

Twenty-nine supernatants from the partially purified FcR immunization contained Ig capable of binding U937 cells. Of these, 12 were of the IgG2a subclass, 1 was IgG3, 7 were IgG1, 2 were IgM, and 7 were either of mixed subclass or could not be typed. The supernatants of the cultures of cloned cells were then evaluated for their ability to bind to a 72kDa cell surface molecule by an affinity adsorption assay.

Detergent lysates of U937 cells radioiodinated by the chloroglycouril method were incubated, (shown in Figure 1) from left to right, with Sepharose-anti-mIg sensitized with purified murine IgG2a myeloma protein RPC5 (lane 1) or with mab 32 (lane 3); with Sepharose-human IgG (lane 4); or with Sepharose-anti-mIg sensitized with mab IV3 (lane 5). The three samples analyzed in the right hand panel were eluted from Sepharose-anti-mIg sensitized with either intact IgG of mab 32 (lane 6), with Fab fragments of pooled human IgG (lane 7), or with Fab' fragments of mab 32 (lane 8). The immunoadsorbants were washed free of unbound radioactivity and the bound material was eluted in an SDS-containing sample buffer and analyzed by electrophoresis on an SDS-polyacrylamide gel followed by autoradiography. Adjacent lanes not shown but marked on the lateral margins of the autoradiograph contained radioiodinated bovine albumin (68kDa) and rabbit muscle actin (43kDa). Lane 2 contains a mab of the IgG2a subclass.

of the two isoelectric focusing patterns with some of the p72 molecules appearing dimmer than in lanes 1-3. most likely because the p72-ligand bond resists dissociation by urea unlike the p40-ligand bond and unlike antibody-antigen interactions (lanes 1-3). Thus, these data further substantiate the identity of the 72 kDa molecules purified by both FcR ligands and mab 32.

Since IFN-gamma enhances the expression of the high affinity FcR, we used indirect immunofluorescence and flow cytometry to examine the binding of mab 32 to control and IFN-gamma-treated U937 cells. Table 1 shows a 3-fold increment in binding of both mab 32 and a murine IgG2a myeloma protein to IFN-gamma-induced U937 cells. We also determined whether hIgG interferes with the binding of mab 32 to the FcR of U937 cells. AS seen in Table 1, hIgG significantly blocked the binding of mIgG2a to the FcR of U937 while the binding of mab 32 was unaffected. This suggests that mab 32 binds to the 72kDa FcR at a site distinct from the ligand binding site.

Table 1

Binding of Mab32 to Control- and IFN Treated U937 Cells

First Antibody	Mean Fluorescence Intensity			
	U937 without IFN		U937 with IFN	
	no hIgG	hIgG	no hIgG	hIgG
P3(mIgG1)	39±1	46±4	55±1	52±6
Mab32(mIgG1)	159±4	150±6	423±8	410±6
RPC5(mIgG2a)	186±16	47±2	537±8	78±4

$5x10^5$ cells from triplicate cultures of U937 cells grown 48 hours with or without 100 IRU/ml IFN were incubated for 2 hours at 4°C in 60 ul RPMI-1640 containing BSA (2mg/ml) and 40 ug/ml IgG fraction of the mIgG1 myeloma P3, and MIgG2a myeloma RPC5 (Litton Bionetics) or Mab32. Replicate mixtures contained 4 mg/ml hIgG to block the FcR binding site. After 3 washes (1 ml cold PBS/BSA, 1 mg/ml) the cells were incubated 2 hours at 4°C with 100 ug/ml FITC anti-mIg (Boehringer-Mannheim), washed with PBS/BSA and fixed in 1% formalin. The cells were analyzed on an Ortho 50H Cytofluorograf using 300 mW excitation at 488 nm. Results are expressed as mean fluorescence intensity ± SD of triplicate cultures. The mean fluorescence intensity of unstained cells (autofluorescence) was 25±2.

We further quantified the ability of both mab 32 and a ligand, in this case a human IgG1 (hIgG1) myeloma protein (Arr), to inhibit the binding to U937 cells of either ^{125}I-human IgG1 (Arr) or ^{125}I-mab 32. Figure 4 shows the results of these inhibition experiments. Under conditions of saturation and equilibrium, U937 cells were incubated at 4°C with ^{125}I-mouse myeloma IgG2a (UPC 10) or ^{125}I-mab 32 in the presence of varying amounts of unlabeled human IgG1. Bound labeled antibody was separated from free by centrifuging the cells through oil and was quantified by counting the radioactivity associated with the cell pellets. Nonspecific binding measured in the presence of a great excess (100 fold) of unlabeled antibody was subtracted from total binding to give specific binding. Percent inhibition, calculated as described in Materials and Methods, was plotted versus the concentration of inhibitor protein. Nonspecific binding was 6-8% of total binding.

As seen in figure 4, human IgG at concentrations found in human serum (10-15 mg/ml) does not inhibit the binding of mab 32 to Fc receptors on U937 cells. On the other hand, if a ligand which binds to the Fc receptor through the ligand's Fc region is used, serum levels of IgG inhibit the binding by more than 95%. In figure 4, the mouse myeloma IgG 2a designated UPC-10 was used as the ligand. Identical inhibition by human IgG has also been demonstrated in experiments using human IgG1 as the ligand. We conclude that the binding of mab 32 to cells does not interfere with ligand binding to the FcR binding site and that ligand binding does not inhibit mab 32 binding.

The types of cells which bear the epitope recognized by mab 32 were evaluated by indirect immunofluorescence and flow cytometry and data are presented in Table 2. It is apparent that mab 32 binds to those cells which are known to bear the high affinity IgG FcR, namely U937, HL60, and monocytes. Lymphocytes were negative as were the B cell lines Raji and Daudi, and the T cell lines Molt4 and Jurkat. Some samples of neutrophils showed low level binding of mab 32. Figure 5 shows the fluorescence intensity of cells stained with mab 32. Each panel displays histograms of fluorescence intensity vs cell

First, mab 32 is of the IgG1 subclass. This murine IgG subclass has been found incapable of binding to the high affinity FcR. See e.g. Anderson, C. L. and Abraham, G. N. (1980) J . Immunol. 125: 2735. Of the seven IgG1 mabs with anti-U937 activity derived from the fusion described above, four of them adsorbed only a 110kDa molecule from lysates of surface radioiodinated U937 cells. Thus, adsorption of the 72kDaFcR is not a general property of IgG1 proteins. (Of the two remaining IgG1 mabs, one adsorbed small amounts of a 72kDa molecule and the other adsorbed both a 72kDa and a 110 kDa molecule; these have not yet been further investigated.) Nevertheless, to eliminate the possibility that mab 32 was a variant IgG1 which bound through its Fc region to the FcR, we tested the capacity of Fab' fragments of mab 32 to adsorb the 72kDa FcR and found that binding occurs independently of the Fc portion of the mab (Fig. 1).

Second, our data show that the 72kDa molecule adsorbed by mab 32 is the same molecule identified as the high affinity FcR by several criteria previously described. See Anderson, C. L. (1982) J. Exp. Biol. 156 : 1794. Not only do the molecules appear identical by polyacrylamide gel electrophoresis in SDS (Figure 1) but the isoelectric focussing patterns of the two molecules are the same as well (Figure 3). The marked heterogeneity of charge of this molecule has been ascribed to terminal sialic acid residues. The preadsorption experiments shown in Figure 2 also support the contention that the 72kDa molecules bound by both ligand and mab 32 are identical. Either ligand or mab 32 is capable of removing the same 72kDa molecule from detergent solution such that it is no longer available to the other for adsorption.

Third, the data indicate that mab 32 binds to a site on the 72kDa FcR distinct from the site where ligand binds (Figure 4, Table 1). This observation constitutes direct evidence that in fact mab 32 is not binding to the receptor as a ligand, for if it were it should inhibit ligand binding. The capacity of mab 32 to bind the ligand-occupied FcR should prove useful in a number of circumstances involving detection of the receptor in the presence of ligand. To date this has been impossible.

Fourth, it is quite clear from Table 2 that the only cells which bear the epitope recognized by mab 32 are those which bear the 72kDa high affinity FcR, namely, monocytes, HL60 cells and U937 cells. This correlation is further evidence that mab 32 is directed against the high affinity FcR. Neutrophils, according to the data of Table 2, are the only other cells capable of binding mab32, but the extent of binding is so low as to be equivocal. Given observations that IFN-gamma induces the expression of this high affinity FcR on neutrophils, it is conceivable that the neutrophils of normal subjects show subtle evidence of induction of this receptor.

Anti-Fc Receptor - Anti-Target Cell Antibody Hetero-aggregates Mediate Human Monocyte ADCC

The IgG1 monoclonal antibody 32.2, raised against the 72 kd monocyte high affinity Fc receptor was used to examine the role of this receptor in ADCC. Whole 32 or its Fab fragments were cross-linked to Fab fragments of rabbit anti-chicken red blood cells (cRBC) using the agent SPDP. The resulting heteroaggregates (32 x Fab anti cRBC) mediated monocyte and U937 cytotoxicity against cRBC. See Figure 6. The covalent association between the anti-Fc receptor and anti-target Fab was found necessary in order for ADCC to occur, since non-cross linked mixtures of 32 and Fab anti-cRBC did not promote ADCC. See Figure 6. U937 cells did not perform appreciable levels of ADCC unless stimulated with IFN-gamma; but ADCC for these cells was stimulated 3 fold with IFN-gamma. (See Figure 7) In contrast, unstimulated human, peripheral blood monocytes (PBM) were able to kill cRBC in the presence of 32 x Fab anti-CE heteroantibodies, and cytotoxicity was increased by IFN-gamma (Figure 8). A control heteroantibody of Fab 32 x Fab anti-Streptococcus mutans did not stimulate control or IFN-gamma treated monocytes or lyse cRBC targets. See Figure 8. 32 x Fab anti-cRBC promoted cell lysis by U937 cells was not inhibited by high levels of blocking IgG1, while cytotoxicity mediated by rabbit anti-cRBC antibody was readily inhibited by IgG1, both with IFN-gamma treated and untreated U937 cells. See Figure 9. Fab 32 x Fab anti-cRBC-promoted killing by human PBMs, with or without IFN-gamma treatment, was not inhibited by increasing levels of blocking IgG1. Rabbit anti-cRBC antibody promoted killing was quickly inhibited by IgG1 regardless of IFN-gamma induction. See Figure 10.

In an attempt to define the cell surface determinants on human monocytes that act as trigger molecules for cytotoxicity, hybridoma cells (HC) which produced antibody directed to various human monocyte surface molecules were selected for high expression of surface Ig and used directly as target cells. Hybridoma cells expressing surface Ig directed to the high-affinity FcgR were efficiently killed by human moncytes, whereas hybridoma cells expressing surface Ig directed to other molecules present on the monocyte membrane were not lysed. Thus, FcgRI, when appropriately triggered, specifically initiates monocyte-mediated cytolysis of tumor target cells. (Graziano and Farger. 1987 J. Immun 138 945-950).

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

Claims

1. A human Fc receptor-specific monoclonal antibody, the binding of which to Fc receptor is not blocked by human immunoglobulin G.

2. A monoclonal antibody which binds, through its antigen binding region, specifically to the high affinity Fc receptor for IgG on human monocytes, the antibody binding site on the receptor being distinct from the ligand binding site for Fc.

3. A monoclonal antibody which:

a. binds specifically to an epitope of the high affinity Fc receptor for IgG on human monocytes, the epitope being distinct from the ligand binding site for Fc of the receptor;

b. is capable of binding to IgG-occupied Fc receptor; and

c. is not blocked from binding to the receptor by human IgG.

4. Monoclonal anti-FcRI antibodies selected from the group consisting of mab 32, mab 22, mab 44, mab 62 and mab 197.

5. A bifunctional antibody or heteroantibody, comprising:

a. at least one antigen binding region derived from an anti-Fc receptor antibody, the binding of which to human Fc receptor is not blocked by human immunoglobulin G; and

b. at least one antigen binding region specific for a target epitope.

6. A bifunctional antibody or heteroantibody according to claim 5, wherein the anti-Fc receptor antibody is specific for the high affinity Fc receptor for human Ig.

7. A bifunctional antibody or heteroantibody according to claim 5 or claim 6, wherein the anti-Fc receptor antibody is selected from the group consisting of mab 32, mab 22, mab 44, mab 62, mab 197 and anti-FcRI antibody 62.

8. A bifunctional antibody or heteroantibody according to claim 5, 6 or 7, wherein the target epitope is that of a cancer cell, that of an infectious agent, or that of an antibody-producing cell.

9. A bifunctional antibody or heteroantibody, comprising:

a. at least one antigen binding region derived from an antibody which

i. binds specifically to an epitope of the high affinity Fc receptor for IgG on human monocytes, the epitope being distinct from the ligand binding site for Fc of the receptor;

ii. is capable of binding to IgG-occupied Fc receptor; and

iii. is not blocked from binding to the receptor by human IgG; and

b. at least one antigen binding region specific for a target cell.

10. A bifunctional antibody or heteroantibody of claim 9, wherein the target cell is selected from the group consisting of a cancer cell, an infectious agent, an IgE-producing cell and an autoimmune cell.

11. A heteroantibody comprising:

a. an antibody or antibody binding fragment specific for Fc receptor for IgG on human monocytes, the binding of which to the human Fc receptor is not blocked by human immunoglobulin G;

b. an antibody or antibody binding fragment specific for a target cell.

12. An antibody according to claim 11, wherein the antibody or antibody binding fragment for Fc receptor is specific for the high affinity Fc receptor.

13. An antibody according to claim 11 or claim 12, wherein the target cell is selected from the group consisting of a cancer cell, an infectious agent, an IgE producing cell and an autoimmune cell.

14. An antibody according to claim 9 or claim 12, wherein the anti-Fc receptor antibody is selected from the group consisting of mab 32, mab 22, mab 44, mab 62 and mab 197.

15. A bifunctional antibody or heteroantibody comprising:

a. at least one antigen binding region derived from mab 32; and

b. at least one antigen binding region derived from an antibody specific for a target cell, said cell for example being a cancer cell.

16. A target-specific effector cell, comprising:

a. an effector cell which expresses receptor for the Fc portion of IgG;

31. An immunoassay according to claim 28 or claim 29, wherein the anti-FcRI antibody is selected from the group consisting of mab 22, mab 32, mab 44, mab 62 and mab 197.

32. The antibodies claimed in any of claims 1 to 15, the effector cells claimed in any of claims 16 to 21, or the targeted macrophage claimed in claim 22 or claim 23, for use as diagnostic agents or therapeutic agent.

33. Methods of making (a) any of the antibodies claimed in any of claims 1 to 15, and (b) any of the cells or macrophages claimed in any of claims 16 to 23, substantially as described in the accompanying specification.

34. A method of targeting a human effector cell, for example a human macrophage which expresses Fc receptor to produce a target-specific effector cell comprising linking an antigen binding region of an antibody specific for a target cell to the effector cell through an antigen binding region derived from an anti-Fc receptor antibody, which binds Fc receptor without being blocked by human IgG, the anti-Fc receptor antibody optionally being selected from mab 32, mab 22, mab 44, mab 62 and mab 197, and the target cell for example being a cancer cell, an infectious agent, an IgE-producing cell or an autoimmune cell.

FIGURE 1

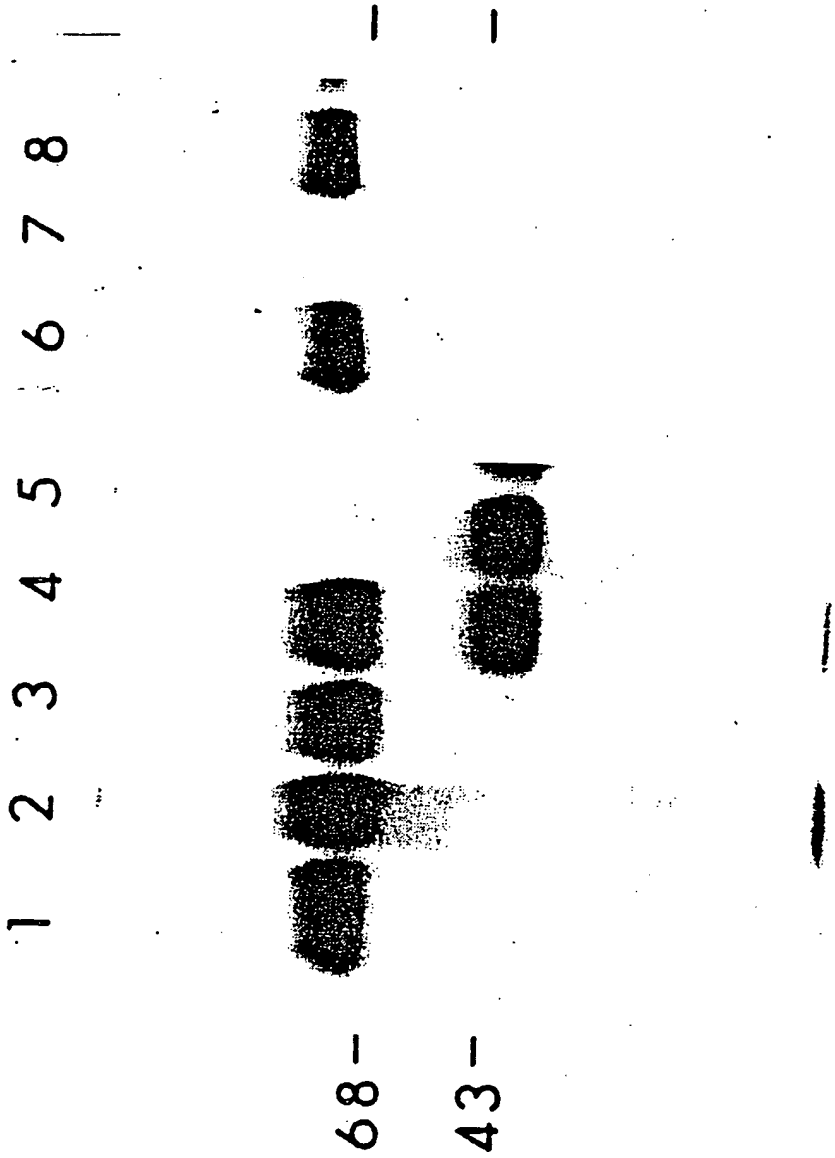

FIGURE 2

Lane	Preclearing Adsorbant	Final Adsorbant	Band Density	% Depletion
1	mIgG1	mIgG2a	310	---
2	mIgG1	32	217	---
3	mIgG2a	32	23	89
4	32	mIgG2a	85	73
5	32	32	42	81
6	mIgG2a	mIgG2a	23	93

FIGURE 3

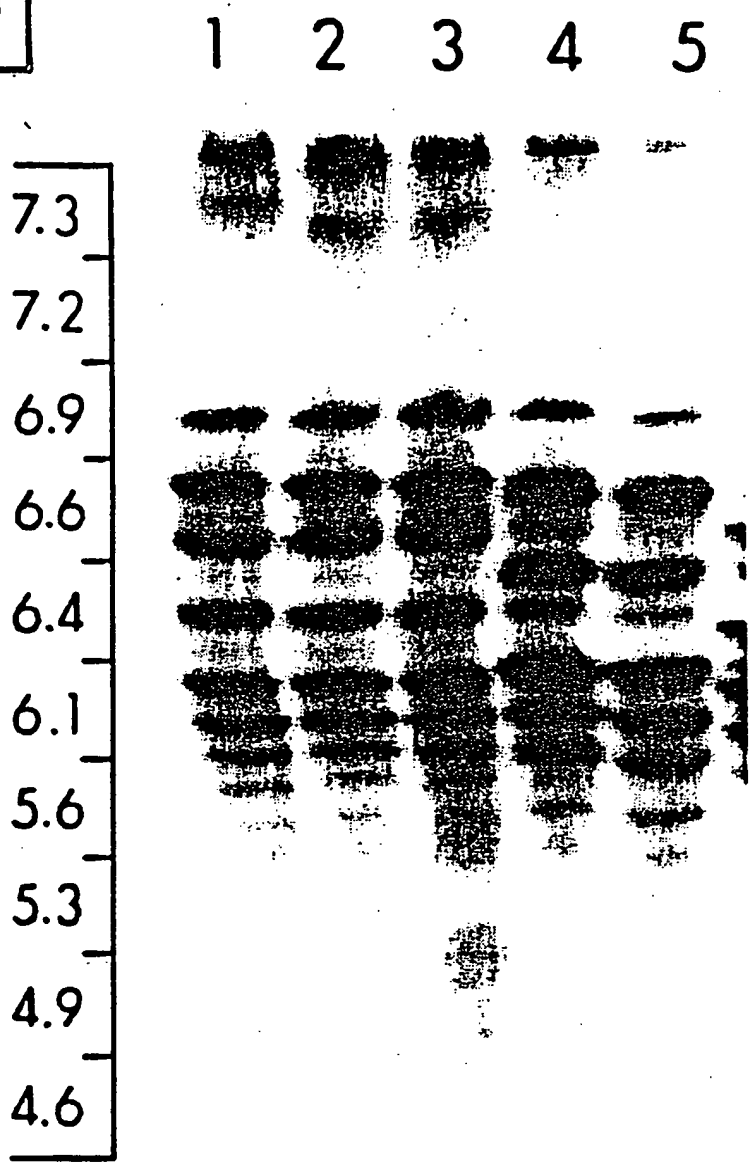

FIGURE 4

Fig. 4. Binding of MAb 32 to U937 Cells

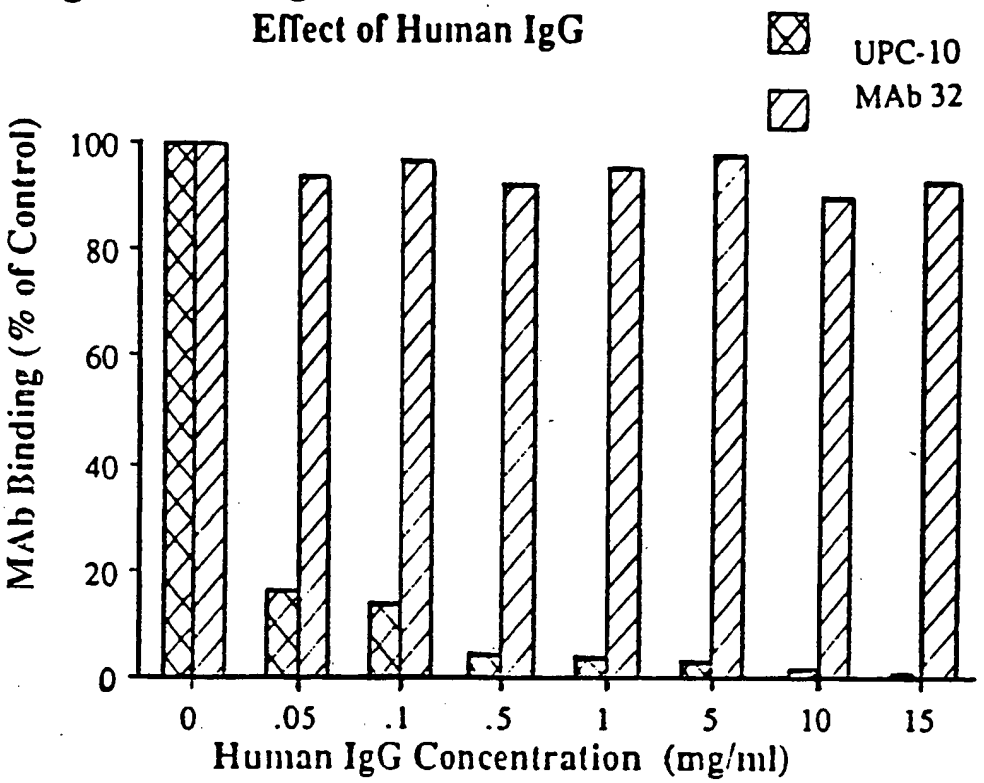

FIGURE 4B

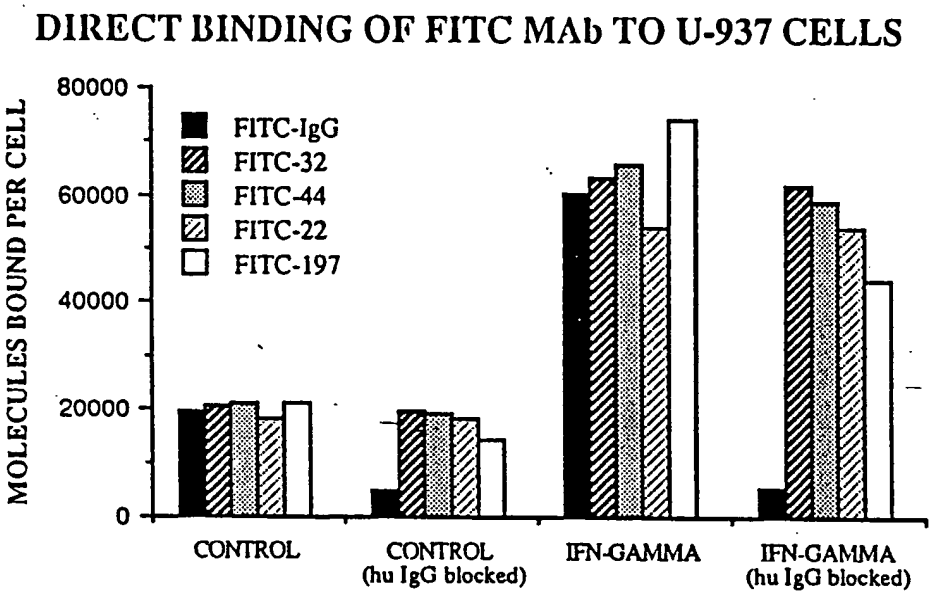

FIGURE 5

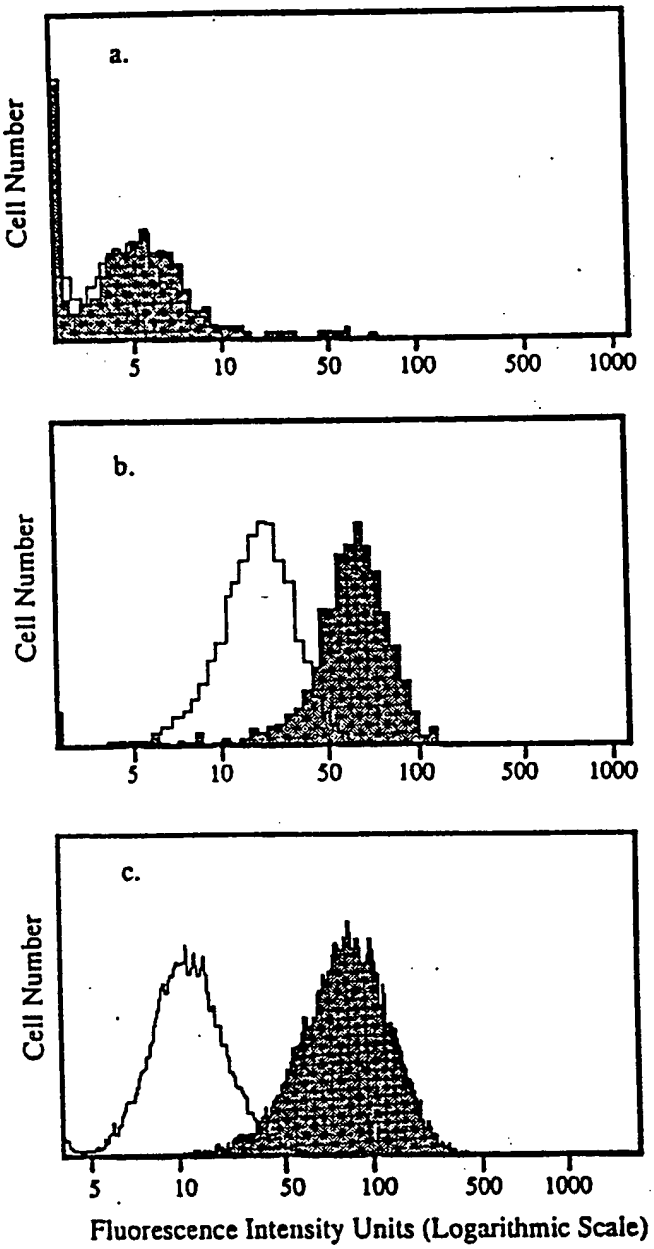

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FIGURE 6

ADCC OF cRBC BY IFN-γ-TREATED U937 CELLS

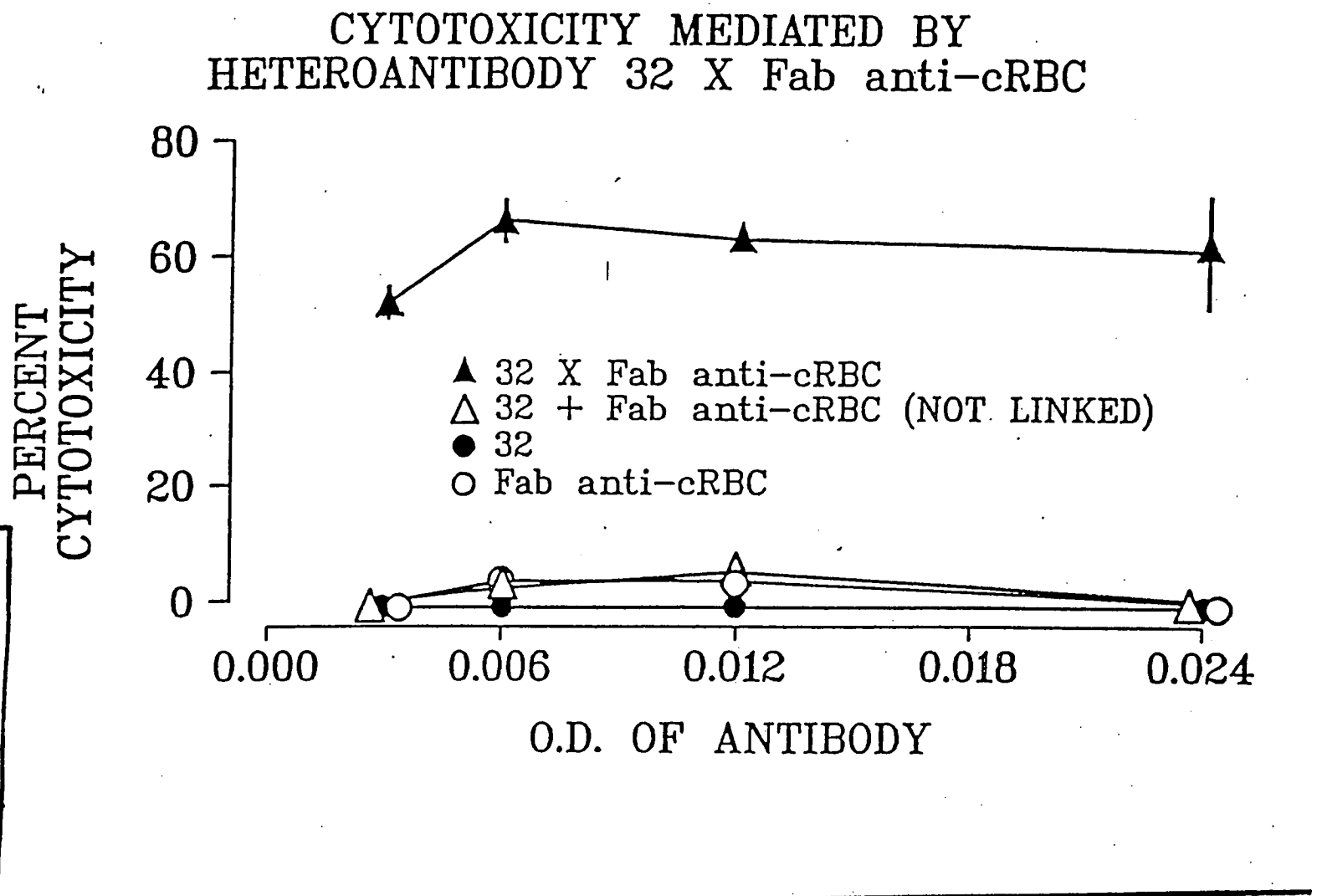


FIGURE 7

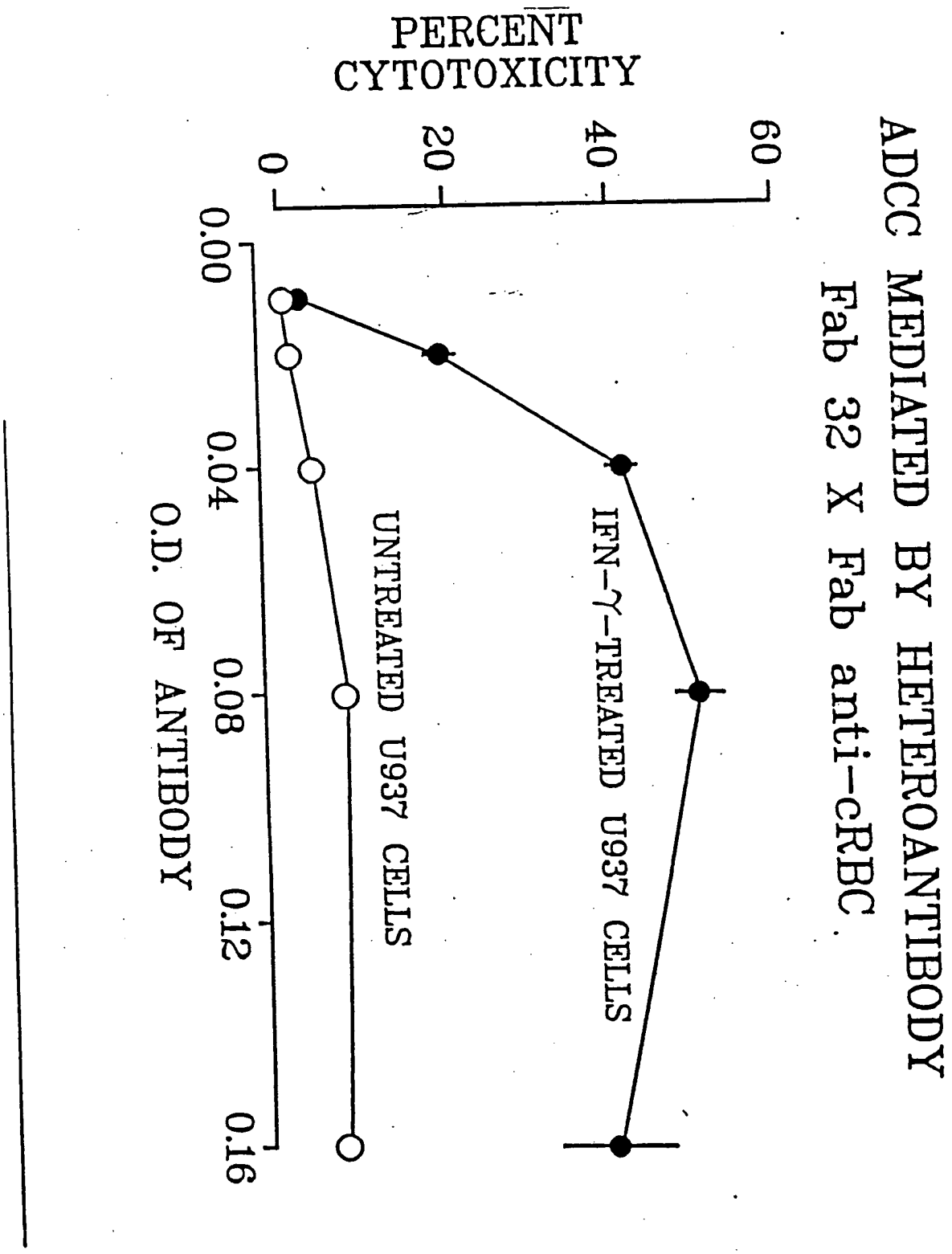


FIGURE 8

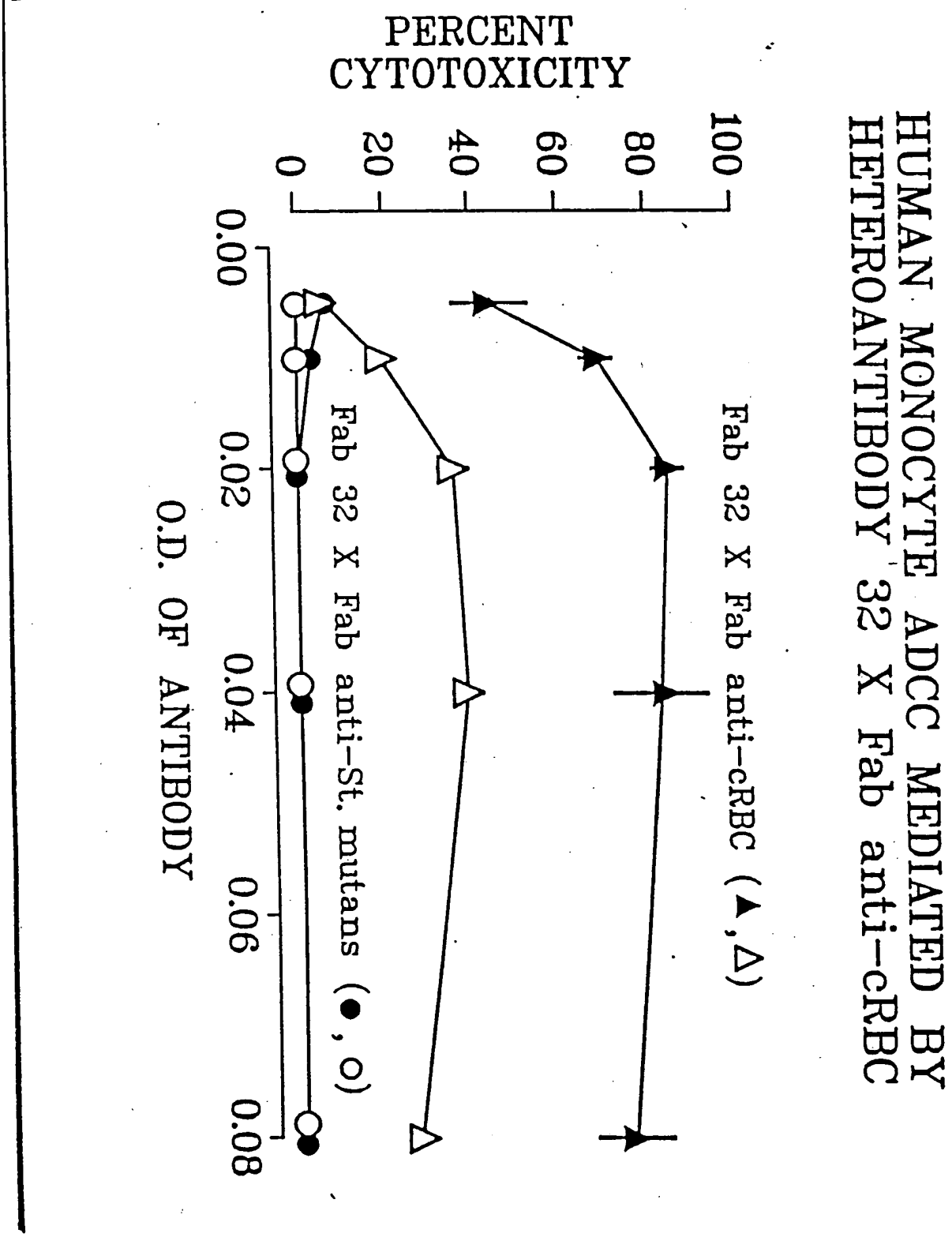


FIGURE 9

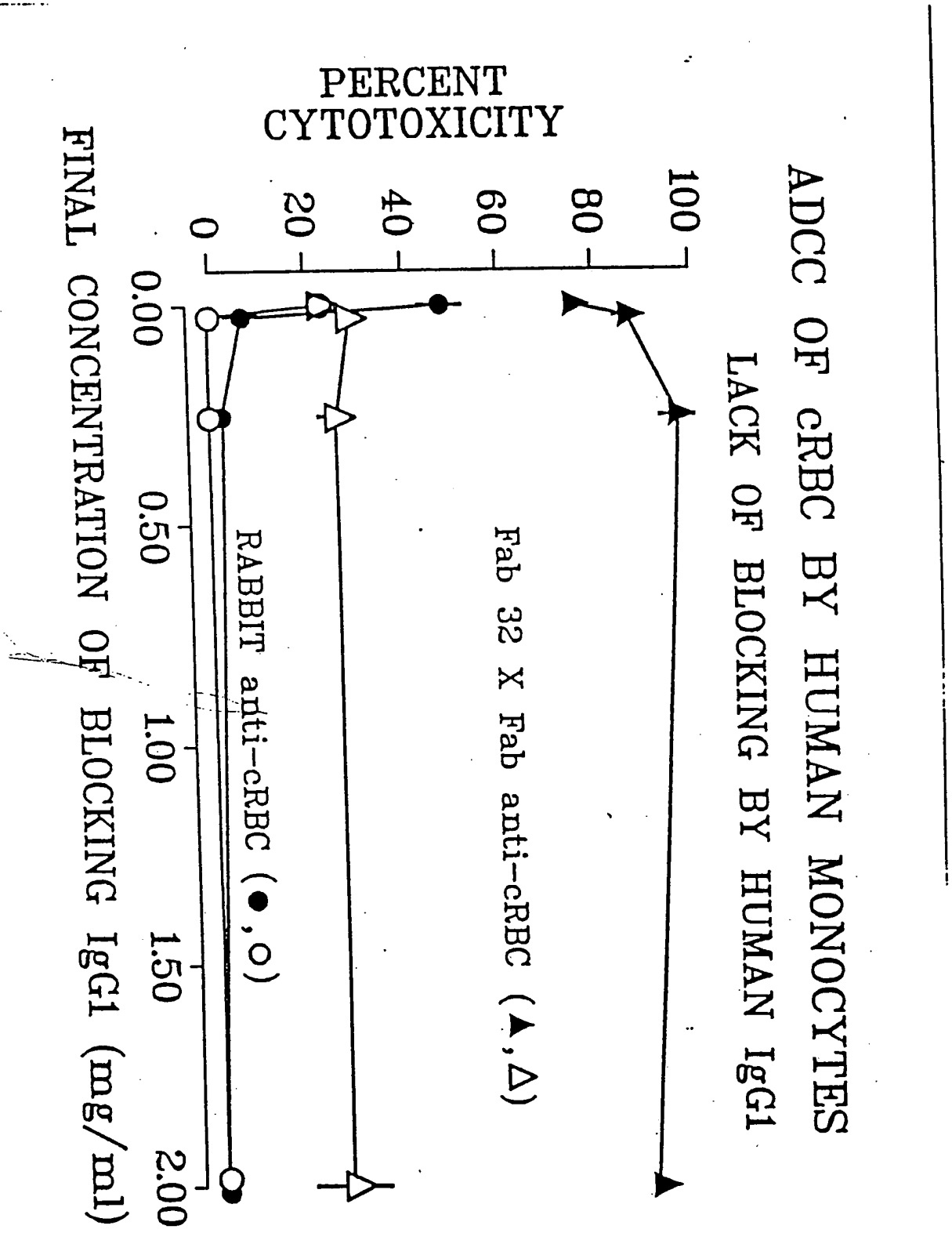
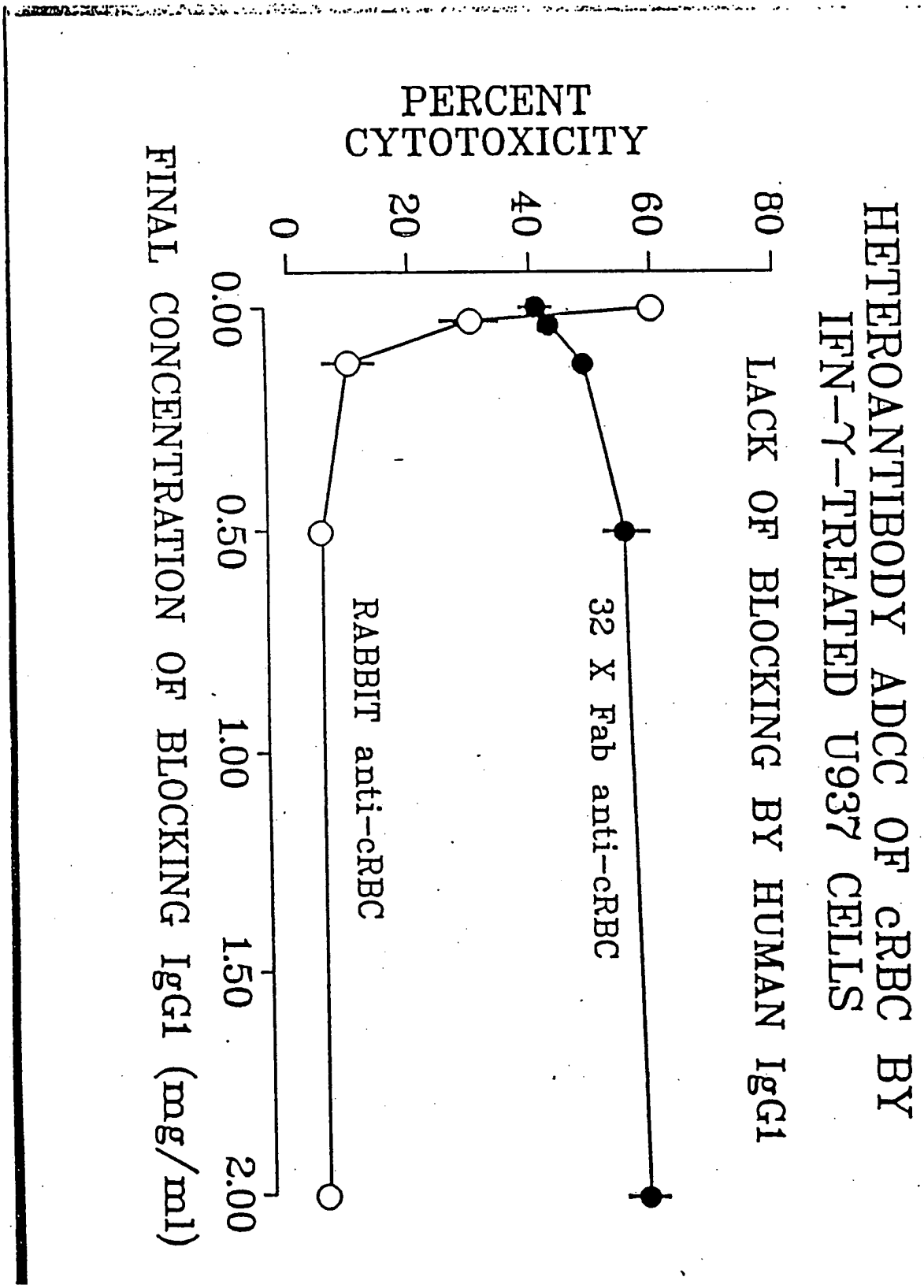


FIGURE 10



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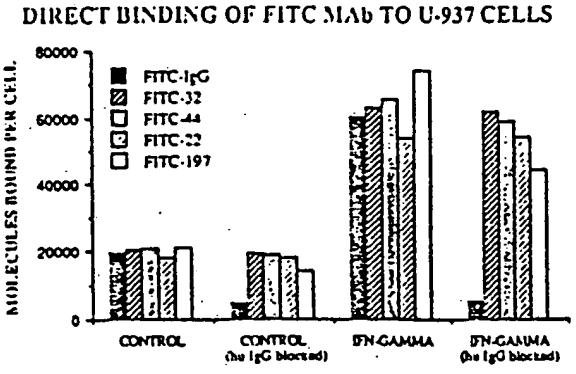
(54) Monoclonal antibodies to Fc receptors for immunoglobulin G on human mononuclear phagocytes; bifunctional antibodies; target specific effector cells; targeted macrophages; and immunoassays.

(57) A human Fc receptor-specific monoclonal antibody is disclosed together with its mode of preparation. Binding of the antibody to Fc receptor is not blocked by human immunoglobulin G. The antibody binds to the high affinity Fc receptor for IgG on human monocytes at a receptor binding site distinct from the ligand binding site for Fc.

A bifunctional antibody or a heteroantibody has an antigen binding region derived from an anti-Fc receptor antibody and an antigen binding region specific for a target epitope or cell: such antibody may target a macrophage when it is bound to surface Fc receptors of the macrophage.

A target-specific effector cell expresses receptor for the Fg portion of IgG, has one antigen binding region derived from an anti-Fc receptor antibody and another specific for a target cell, and the aforesaid bifunctional or hetero-antibody is bound to the Fc receptor of the effector cell: such effector cell can be used in the therapy of cancers, allergies, infectious and autoimmune diseases, and in immunoassays.

FIGURE 4B



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CLAIMS INCURRING FEES

The present European patent application comprised at the time of filing more than ten claims.

☐ All claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for all claims.

☐ Only part of the claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims and for those claims for which claims fees have been paid.

namely claims:

☐ No claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims.

X LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirement of unity of invention and relates to several inventions or groups of inventions,
namely:

1) claims 1-4,28,32,33: Monoclonal antibody to human Fc-receptor and immunoassay employing this antibody.

2) claims 5-15: Bifunctional or hetero-antibody.

3) claims 16-27,34: Targetspecific effector cell and method of targetting human effector cells.

4) claims 29-31: An immunoassay for quantifying g-IFN.

☐ All further search fees have been paid within the fixed time limit. The present European search report has been drawn up for all claims.

☒ Only part of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the inventions in respect of which search fees have been paid.

namely claims: 1-15,28,32,33

☐ None of the further search fees has been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims.

namely claims:

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
Y	JOURNAL OF EXPERIMENTAL MEDICINE, vol. 160, December 1984, pages 1686-1701, New York, NY, B. KARPOVSKY et al.:"Production of target specific effector cells using hetero-cross-linked aggregates containing anti-target cell and anti-Fcg receptor antibodies" * whole document * ---	5-15	
A	EP - A- 68763 (THE BOARDS OF REGENTS UNIVERSITY OF TEXAS SYSTEM) * claims 1-13 * ---	5-15	
A	EP - A-96463 (E.I. DU PONT DE NEMOURS) * pages 16-23, claims 1-10 * ---	5-15	TECHNICAL FIELDS SEARCHED (Int. Cl.4)

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